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ORIGINAL ARTICLE

The chromosome content and genotype of two wheat cell lines and of their somatic fusion product with oat

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Abstract Somatic hybridization seeks to genetically combine phylogenetically distant parents. An effective system has been established in bread wheat (*Triticum aestivum* L.) involving protoplasts from a non-totipotent cell line adapted to in vitro culture (T_1) in combination with totipotent protoplasts harvested from embryogenic calli (T_2). Here, we report the karyotype and genotype of T_1 and T_2. Line T_1 carries nine A (A-genome of wheat), seven B (B-genome of wheat) and eight D (D-genome of wheat) genome chromosomes, while T_2 cells have 12 A, 10 B and 12 D genome chromosomes. Rates of chromosome aberration in the B- and D-genomes were more than 25%, higher than in the A-genome. DNA deletion rates were 55.6% in T_1 and 19.4% in T_2, and DNA variation rates were 8.3% in T_1 and 13.9% in T_2. Rate of DNA elimination was B- > D- > A-genome in both T_1 and T_2. The same set of cytological and genetic assays was applied to a derivative of the somatic fusion between protoplasts of T_1, T_2 and oat (*Avena sativa* L.). The regenerant plants were near euploid with respect to their wheat complement. Six wheat chromosome arms—4AL, 3BS, 4BL, 3DS, 6DL and 7DL—carried small introgressed segments of oat chromatin. A genotypic analysis of the hybrid largely confirmed this cytologically-based diagnosis.

Keywords Chromosome content and genotype · Common wheat · Oat · Somatic hybrid

Abbreviations

T	*Triticum aestivum* cv. Jinan 177
T_1	Jinan 177 suspension cells
T_2	Jinan 177 embryogenic calli
SSR	Simple sequence repeats
GISH	Genomic in situ hybridization
FISH	Fluorescence in situ hybridization

Introduction

Numerous studies have been focused on analysis of somaclonal variation in vitro (Larkin et al. 1984; Lee and Phillips 1988; Pershina et al. 2003). The initial reports were concerned with determining changes in the number, structure, and morphology of the chromosomes, such as chromosome banding patterns (Larkin et al. 1984; Lee and Phillips 1988), and later studies examined variations in genomic DNA caused by mutations, the loss and rearrangement of genes, gene silencing and transposons or reverse transposons in subculture (Shaked et al. 2001; Polanco and Ruiz 2002; James and Reiner 2007). However, little was known about the content and genotype of cultured cells of allohexaploid wheat (*Triticum aestivum* L.) that had been grown for different periods of time.

Wheat (*T. aestivum* L., $2n = 42$) is an important cereal. The genetic variability for some important traits, such as high quality, diseases and stress resistance, is limited in the cultivated wheat germplasm. Related or distant species of wheat are an important reservoir of useful genes (Liu et al.

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2005). There is thus an urgent need to broaden the wheat gene pool by introgressing genes for such traits from diverse cereal species. This kind of alien introgression line created can increase the genetic variation in wheat species. However, the sexual route with low crossability in achieving this limited the transfer of such genes (Wang et al. 2004; Liu et al. 2005). Somatic hybridization provides the possibility of overcoming sexual incompatibility of wheat with remote cereals (Xia et al. 2003). This technique has been used with some success as a means of introgressing genes from related grass and cereal species (Xia et al. 2003; Wang et al. 2005; Zhou and Xia 2005). However, when the donor species was only distantly related to wheat, fusions often failed to progress beyond undifferentiated calli or albino plants (Xiang et al. 1999; Li et al. 2001; Yue et al. 2001). In an attempt to overcome the difficulty of regenerating hybrids between distantly related species, a three-parent fusion system has been elaborated (Xiang et al. 2003a, 2004; Xu et al. 2003), based on two complementary wheat cell lines T_1 and T_2. The former is a non-totipotent cell line growing in long-term cell suspension, and the latter are totipotent cells taken from embryogenic calli (Xiang et al. 2003b). The third "parent" is an exotic donor species, the protoplasts of which are irradiated before fusion. Using this system, Xiang et al. (2003a) were able to regenerate a set of putative wheat/oat (*Avena sativa* L.) somatic hybrids and employed a combination of cytological and genetic assays to demonstrate the presence of a variable number of wheat/oat recombinant chromosomes. However, the contents and genotypes of the two wheat cell lines and their somatic fusion products with oat were unknown. This was critical for understanding the mechanism of the regeneration of hybrid plants.

In this work, our goal was to investigate the chromosome elimination and variation of T_1 and T_2, as well as the relationship between the genetic complementation of T_1, T_2, oat and somatic hybrid plant regeneration.

Materials and methods

Plant materials

Wheat protoplasts derived from two types of cell lines T_1 and T_2 of *T. aestivum* L. cv. Jinan 177, and oat protoplasts came from embryogenic calli *A. sativa* L. cv. Mapur. The T_1 were harvested from a non-regenerable long-term (>10 years) cell suspension, and the T_2 from 2-year-old embryogenic calli with regeneration frequency of 75% (number of calli which can differentiate to plants/number of calli analysed, %) (Xiang et al. 2003a). The oat cell line was 1.5-year-old embryogenic calli (Xiang et al. 2003a). The T_1 and T_2 lines were subcultured on MB liquid and solid media (Xia and Chen 1996) with 2.0 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D). Protoplasts were isolated from T_1 suspension after 3 days of subculture, T_2 and oat embryogenic calli were isolated 7 days after subculture according to the method of Xia and Chen (1996). After washing in 0.6 M mannitol and 5 mM $CaCl_2$, oat protoplasts were transferred onto 3.5 cm Petri dishes in a thin layer and irradiated with UV light at an intensity of 300 $\mu W/cm^2$ for 1 min before they were fused with T_1 and T_2 (Xiang et al. 2003a). T_1 and T_2 protoplasts were combined at the ratio of 1:1 and mixed in equal volumes with UV-treated protoplasts of oat. The fusion was carried out according to the improved PEG method (Xia and Chen 1996). When the fusion clones grew to small cell lines of 1.5–2.0 mm in diameter, they were transferred to the MB medium with 1 mg/L 2,4-D for proliferation. After subculture for 1–2 months, proliferating calli from the cell lines were moved to the MB medium containing 0.5 mg/L IAA and 0.5 mg/L zeatin for regeneration. Regenerated plantlets were transferred to seedling-strengthening MB medium containing 1–2 mg/L multi-effect triazal (MET) and 0.5 mg/L NAA for strengthening and rooting. All the regenerated plants from four hybrid clones resembled wheat, but only plants of clone no. 94-1 can grow into soil, and then tassel normally (Xiang et al. 2003a). In this experiment, a lager number of roots from hybrid plants of clone no. 94-1 in different growth stages were analysed. Accessions of *Triticum urartu* Thum, *Aegilops speltoides* Tausch and *Aegilops tauschii* (Cross.) Schmal were kindly provided by the Quality and Resource Institute of the Agriculture Science Academy of China.

Karyotype analysis and in situ hybridization

Chromosome spreads were obtained from T_1 and T_2 cells and from the root tips of the regenerants and germinating seedlings, as described by Xiang et al. (2003b). Karyotypes of cv. Jinan 177 were derived from a sample of ten mitotic metaphase cell spreads. Karyotype classification followed the method of Sears (1969) and was based on the data for Chinese Spring wheat from Gill (1987). The parameters of the karyotypes were based on ten metaphase cell spreads. The arm ratios and the relative distances of the chromosomes from the hybrid cells selected were analysed. For in situ hybridization (ISH) purposes, genomic DNA of cv. Jinan 177, oat, *T. urartu*, *Ae. tauschii*, *Ae. speltoides* and the hybrid regenerants was isolated following Doyle and Doyle (1990). The pSc119.2 and pAs1 plasmids contain, respectively, B and D genome-specific repetitive sequences (Mukai et al. (1993). These were labelled for use as fluorescence ISH (FISH) probes with digoxigenin-11-dUTP, using a nick translation kit, according to the manufacturer's

instructions (Boehringer Mannheim, Germany), as described elsewhere (Wang et al. 2005). Total oat genomic DNA was labelled in the same way to generate a genomic ISH (GISH) probe. The GISH and FISH methodology followed Wang et al. (2005), the former using a ratio of 1:50 labelled oat genomic DNA to unlabelled wheat genomic DNA, and the latter 1:50 labelled pSc119.2 to unlabelled genomic (*T. urartu* + *Ae. tauschii*) DNA, or 1:50 pAs1 to unlabelled genomic (*T. urartu* + *Ae. speltoides*) DNA. In sequential GISH and FISH experiments (pAs1/pSc119.2 and GISH/pAs1/pSc119.2), the initial signal was rinsed before rehybridization with the subsequent probe.

Microsatellite (SSR) analysis

DNA extracted from T_1 cells, T_2 calli and leaf of somatic hybrids and parental lines (Doyle and Doyle 1990) was used as template for the analysis of allelic constitution at 101 SSR loci, following Röder et al. (1998). The marker set covered the centromeric and subtelocentric regions of all the chromosomes present in T_1 and T_2, and the locations selected based on the previous GISH, FISH and karyotype analysis of hybrid. The relative distance (%; the distance from the centromere to SSR locus checked/the length of the arm involved × 100%) was measured according to the genetical map of Röder et al. (1998).

Results

The chromosome content of T_1 and T_2 and cv. Jinan 177

Of the 138 T_1 cells analysed, 119 (86.2%) contained 22–25 chromosomes (Fig. 1a), while 104/124 (83.9%) of the T_2 cells had 34–38 chromosomes (Fig. 1b). Some T_1 and T_2 cells included telocentric chromosomes, acrocentric chromosomes and dicentric chromosomes (see Suppl. Table 1, Fig. 1a, b). One or two chromosome fragments were present in ~87% of both the T_1 and T_2 cells. The karyotype of cv. Jinan 177 conformed to that of standard bread wheat (Gill 1987; Suppl. Table 2, Fig. 1c).

The genome content of T_1 and T_2 cells

FISH preparations based on hybridization with pSc119.2 and pAs1 succeeded in defining the B and D genome content of T_1 and T_2. From a sample of 136 T_1 cells, 82 (~60%) contained seven B genome, and 83 (~61%) contained eight D genome chromosomes (Table 1, Fig. 2a, b); similarly, of the 95 T_2 cells analysed, ~60% had ten B and ~54% 12 D genome chromosomes (Table 1, Fig. 2c, d). The range in the number of B and D genome

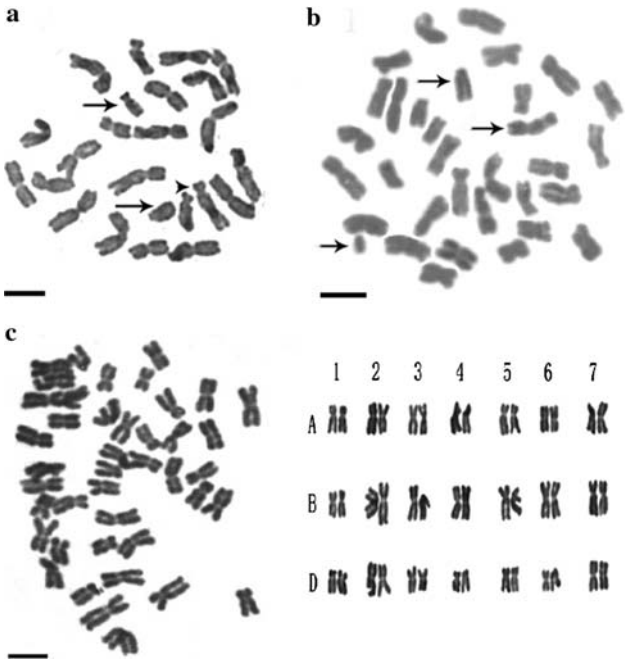

Fig. 1 Mitotic metaphase spreads of T_1 (**a**), T_2 (**b**), cv. Jinan 177 (**c**) and the karyotype of cv. Jinan 177 (**d**). Telocentric and subtelocentric chromosomes indicated by an *arrow*, dicentric chromosomes by an *arrowhead*

chromosomes in T_1 was, respectively, 6–8 and 7–9, and in T_2, 9–11 and 10–12. Chromosomes 2B, 3B, 7B, 3D and 6D were present in most T_1 and T_2 cells, but 4B, 5B and 4D were rare. Chromosome abnormalities were present in, respectively, 27 and 39% of the T_1 B and D genome chromosomes and 31 and 36% of T_2 chromosomes (Table 2). Therefore, many eliminations and rearrangements including duplication of B- and D-genome chromosomes in T_1 and T_2 presented in the calli subculture. Chromosome arm ratios and relative lengths were used to infer the A genome content of T_1 and T_2 cells. Comparing the statistics of karyotype data of T_1, T_2 with cv. Jinan 177, it was revealed that A genome chromosomes of T_1 and T_2 were distributed mainly in 4A and 5A, whereas 3A was the lowest. About 61% of T_1 cells contained nine A genome chromosomes, while ~57% of the T_2 cells contained 12 (Table 1, Fig. 2a, b). The range in A genome chromosome number in T_1 was 7–11, and in T_2 11–15. Non-standard A genome chromosomes were present in 22% of T_1 and 16% of T_2 cells (Tables 1, 2).

Distinguishing between DNA deletion and modification in T_1 and T_2

Simple sequence repeats genotyping was employed to distinguish between DNA deletion and modification in T_1 and T_2 as a result of cell culture. Of the 58 loci assayed, 36 exposed genetic polymorphism between T_1 and T_2 and T

Table 1 Frequency of pSc119.2 and pAs1 hybridization signals in T_1 and T_2 cell lines

DNA probe	Material	Main chromosome no.	Cell no. observed	Signal number and frequency (%)						
				6 (%)	7 (%)	8 (%)	9 (%)	10 (%)	11 (%)	12 (%)
pAs1	T_1	23	45		7 (15.5)	29 (64.4)	9 (20.1)			
		24	58		11 (19.0)	36 (62.0)	11 (19.0)			
		25	33		6 (18.2)	19 (57.6)	7 (21.2)			
	T_2	33	38					8 (21.1)	10 (26.3)	20 (52.6)
		34	25					4 (16.0)	7 (28.0)	14 (56.0)
		35	32					6 (18.2)	9 (28.1)	17 (53.1)
pSc119.2	T_1	23	44	14 (31.8)	25 (56.8)	5 (11.4)				
		24	48	14 (29.2)	30 (62.5)	4 (8.3)				
		25	31	7 (22.6)	19 (61.3)	5 (16.1)				
	T_2	33	19				5 (26.4)	11 (57.9)	3 (15.7)	
		34	23				5 (21.7)	14 (60.9)	4 (17.4)	
		35	15				4 (26.7)	9 (60.0)	2 (13.3)	

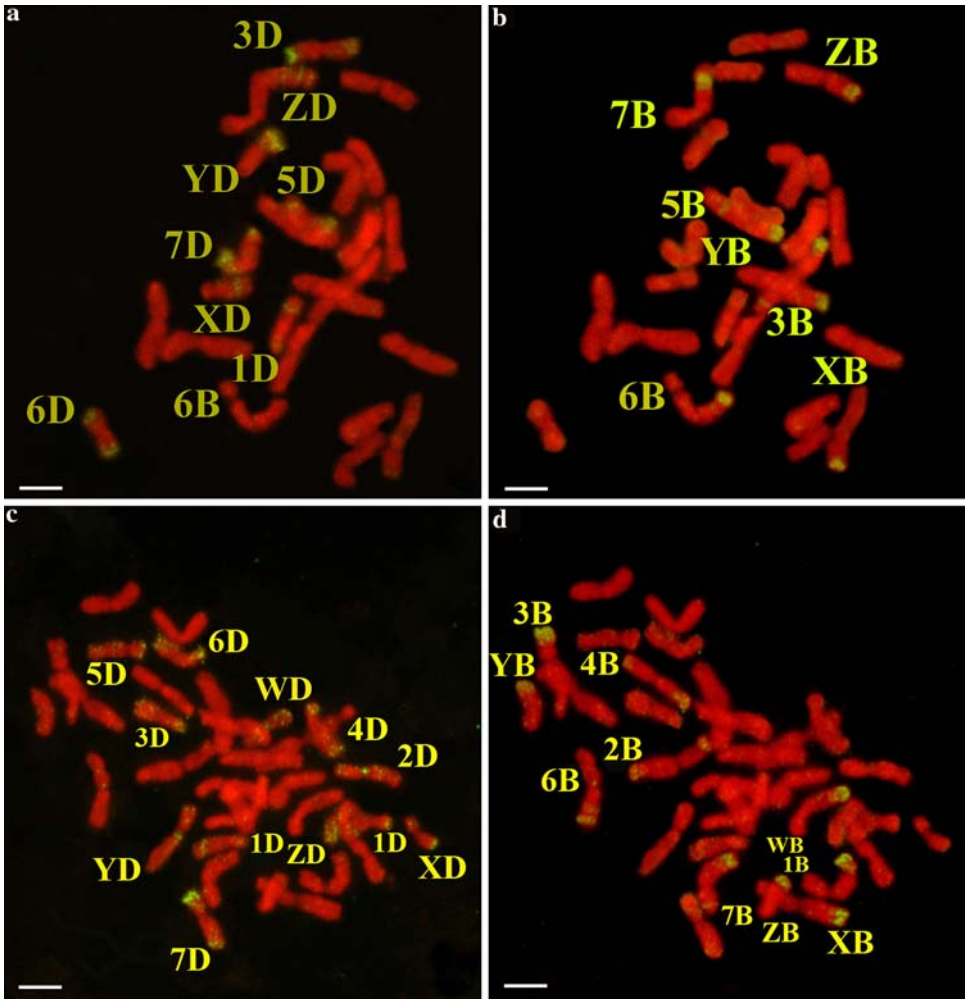

Fig. 2 FISH profile of the mitotic metaphase chromosomes. **a** and **b** B and D genome chromosomes of T_1 identified by their pSc119.2 and pAsI patterns. **c** and **d** B and D genome chromosomes of T_2 identified by their pSc119.2 and pAsI patterns. *Scale bars* 10 μm

(cv. Jinan 177), and the remainder were non-informative (Table 3, Fig. 3). Of the 36 informative markers, 13 showed same patterns among T, T_1 and T_2, 15 between T_1 and cv. Jinan 177 and 25 between T_2 and cv. Jinan 177 (Table 3). The rates of DNA deletion were 55.6% (20/36) in T_1, such as in Xgwm160 and Xgwm428 (Fig. 3), and

Table 2 Chromosome constitution of the T_1 and T_2 lines

A-genome chromosome	T_1 No. of chromosome (%)	T_2 No. of chromosome (%)	B-genome chromosome	T_1 No. of chromosome (%)	T_2 No. of chromosome (%)	D-genome chromosome	T_1 No. of chromosome (%)	T_2 No. of chromosome (%)
1A	8 (8.89)	5 (9.09)	1B	10 (8.93)	7 (8.33)	1D	14 (11.86)	12 (6.78)
2A	9 (10.00)	7 (12.73)	2B	16 (14.29)	8 (9.52)	2D	9 (7.63)	26 (14.69)
3A	4 (4.44)	2 (3.64)	3B	16 (14.29)	16 (19.05)	3D	14 (11.86)	21 (11.86)
4A	21 (23.33)	9 (16.36)	4B	4 (3.57)	4 (4.76)	4D	5 (4.24)	6 (3.39)
5A	13 (14.44)	12 (21.82)	5B	8 (7.14)	3 (3.57)	5D	7 (5.94)	15 (8.47)
6A	7 (7.78)	5 (9.09)	6B	12 (10.71)	8 (9.52)	6D	19 (16.10)	18 (10.17)
7A	8 (8.89)	6 (10.91)	7B	16 (14.29)	12 (14.29)	7D	4 (3.39)	16 (9.05)
XA	20 (22.22)	9 (16.36)	XB	30 (26.79)	26 (30.95)	XD	46 (38.98)	63 (35.59)
Total	90 (100)	55 (100)	Total	112 (100)	84 (100)	Total	118 (100)	177 (100)

19.4% (7/36) in T_2, such as in Xgwm335 (Fig. 3) and Xgwm273, and 16.7% (6/36) in both T_1 and T_2 (Table 3). This indicated that the rate of DNA deletion of T_1 was higher than that of T_2. The ratio of DNA modification was 8.3% (3/36) in T_1 with examples such as Xgwm389 (Fig. 3) and Xgwm160 (Fig. 3). In T_2 the ratio of DNA modification was 13.9% (5/36), with examples of Xgwm389, Xgwm335 and Xgwm428 (Table 3, Fig. 3). B-genomic DNA was eliminated at a faster rate than D-genomic DNA, which was deleted faster than A-genomic DNA in T_1 and T_2. Anyway, the rate of DNA deletion was greater in T_1 than in T_2, while the rate of DNA modification was greater in T_2 than in T_1 (Table 3). Therefore, there were many instances of DNA variation at different positions in T_1 and T_2, and within the calli of subcultures of wheat.

Introgression of oat chromatin into wheat

About 71% of the cells of the hybrid regenerants contained 46–48 chromosomes (mean 47.2), with ~58% containing oat chromosome introgression segments distributed on six wheat chromosomes. Based on sequential ISH experiments, it was possible to identify 12–16 of the B genome and 11–13 of the D genome chromosomes (Table 4, Fig. 4a, b). By comparing their karyotype with that of cv. Jinan 177, 17 A genome chromosomes and three chromosomal abnormalities were detected (Table 4). Almost the whole wheat genome was represented in the hybrid nuclei, along with duplicated, deleted or re-arranged chromosomes. The GISH/FISH analysis identified the presence of oat chromatin on chromosomes 3B and 4B (Fig. 4a, c), and 3D, 6D and 7D (Fig. 4b, c). A further introgression site was identified indirectly on chromosome 4A (Fig. 4c). The arm locations of the six introgression sites were 4AL, 3BS, 4BL, 3DS, 6DL and 7DL (Table 5, Fig. 4). An analysis of the arm ratios of the introgressed chromosomes, and the relative distances between the centromere and the introgression break point are given in Table 5.

Of the 58 SSR loci used to genotype the hybrid regenerants, 32 were amplified in all of the samples. Of the 32 informative markers, only 14 (43.8%) identified differences between hybrid plants and their parents. The alleles from both parents were present at nine of above 14 loci, only the oat allele was amplified at two loci, while at the remaining three loci, the alleles were biparental and novel (Table 6). The relative distance (%) between the centromeres and the SSR loci were measured. SSR bands were located on 4AL, 3BS, 4BL, 3DS, 6DL and 7DL chromosome arms, and the relative distances (%) of fragments ranged from 3.10 to 76.36% (Table 6), in agreement with the results of sequential GISH and FISH (Table 5). Oat alleles were also amplified by primers targeting SSR loci on chromosome arms 3AS, 3AL, 6AL and 7AL, none of which were identified by GISH as sites of introgression (Table 6). This observation is suggestive of the occurrence of many submicroscopic introgression events.

Discussion

By using the pSc119.2 with high repetitive sequences of B-genome from rye (*Secale cereale* L.), and pAs1 with insertion of repetitive sequences of D-genome from *Ae. tauschii* for two-colour FISH, Mukai et al. (1993) established the ideogram of B- and D-genomes and a pair of 4A chromosomes of Chinese Spring wheat. Using N-, C-banding, GISH, FISH and SSR markers in combination with karyotypes data, heterogeneric chromatin in many wheat hybrids were localized into the wheat chromosomes (Jiang et al. 1993; Nagy et al. 2002; Malysheva et al. 2003; Silkova et al. 2006). Our previous result had also confirmed that FISH analysis with pSc119.2 and pAs1, in combination with karyotype data and GISH, could differentiate all

Table 3 SSR profiles of cv. Jinan 177 and T_1 and T_2 cell lines

Primer	Location	The amplification result of wheat and its calli			
		Jinan 177	T_1	T_2	Deletion and modification of genomic DNA in T_1 and T_2
Xgwm359	1AS	T	T	T	
Xgwm47	2AL	T	T	T	
Xgwm674	3AL	a + b	0	a + b	D in T_1
Xgwm369	3AS	a + b	a	a + b	D in T_1
Xwmc258	4AL	a + b	0	0	D in T_1 and T_2
Xwmc262	4AL	a + b	0	a + b	D in T_1
Xgwm160	4AL	a + b	a + N	a + b	D in T_1, M in T_1
Xgwm637	4AL	T	T	T	
Xgwm494	6AL	T	T	T	
Xgwm63	7AL	a + b	0	a + b	D in T_1
Xgwm140	1BL	T	T	T	
Xgwm273	1BS	a + b+c	a	N	D in T_1 and T_2, M in T_2
Xgwm297	3BL	T	T	T	
Xgwm247	3BL	a + b	0	a + b	D in T_1
Xgwm547	3BS	T	T	T	
Xpsp3030	3BS	a + b+c	a + b	a + b	D in T_1 and T_2
Xgwm285	3BS	T	T	T	
Xgwm231	3BS	a + b+c + d	a + b+c	a + b	D in T_1 and T_2
Xgwm389	3BS	a	a + N	a + N	M in T_1 and T_2
Xgwm77	3BS	T	T	T	
Xwmc69	3BS	a + b	0	a + b	D in T_1
Xgwm107	4BL	a + b+c + d+e	a + b+c	a + b+c + d+e	D in T_1
Xgwm165	4BL	a + b	0	a + b	D in T_1
Xgwm368	4BS	T	T	T	
Xgwm335	5BL	a	a	N	D in T_2, M in T_2
Xgwm219	6BL	T	T	T	
Xgwm537	7BS	a + b	0	a + b	D in T_1
Xgwm157	1 DL	T	T	T	
Xgwm337	1 DS	a + b+c	a + b	a + b+c	D in T_1
Xgwm314	3DL	T	T	T	
Xgwm456	3DS	a + b	a + b	a + b+N + N	M in T_2
Xgdm98	6DL	a + b	0	a + b	D in T_1
Xgwm428	7DL	a + b	0	a + b+N	D in T_1, M in T_2
Xgdm46	7DL	a + b	b	a	D in T_1 and T_2
Xpsp3079	7DL	a + b+c + d	a + b+c + N	a + b+c	D in T_1 and T_2, M in T_1
Xgwm437	7DL	a + b	0	a + b	D in T_1
Rate of D (%)	T_1	55.6	Rate of M (%)	T_1	8.3
	T_2	19.4		T_2	13.9

T non-informative; *N* novel allele

a, *b*, *c* the band amplified; *0* no amplification; *D* locus absent; *M* modification type

of the A-, B- and D-genome chromosomes of the cultivar wheat Jinan 177 and localize small donor chromosomes in somatic hybrid of Jinan 177 with *Agropyron elongatum* (Wang et al. 2005). Here, we have extended this analysis to the three-cell fusion system (Xiang et al. 2003a), which has uncovered chromosome content and genotype of two wheat cell lines and of their somatic hybrid plant with oat.

Chromosome loss and variation in the T_1 and T_2 cell lines

Variation in chromosome number and the formation of chromosomal aberrations are common in cells cultured in vitro over many generations (Larkin et al. 1984; Song et al. 2000). Here we have shown that the T_1 cell line has lost

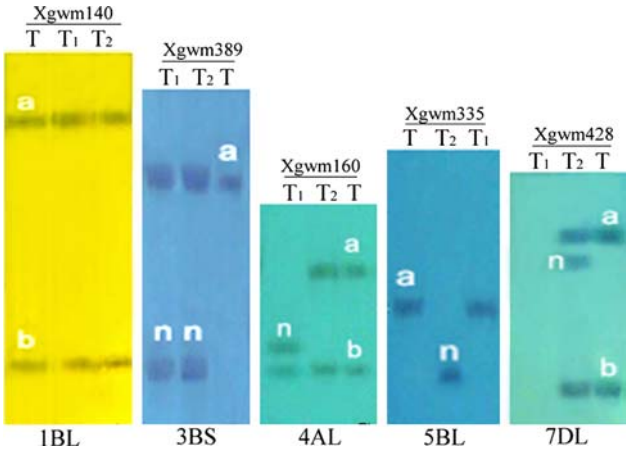

Fig. 3 SSR profiles of cv. Jinan 177 (T) and cell lines T_1 and T_2, based on primers directed to loci on wheat chromosome arms 1BL, 3BS, 4AL, 5BL and 7DL. a, b: cv. Jinan 177 allele; *n* novel allele

about 18, while T_2 has lost only about eight chromosomes (Table 1, Fig. 2), as would be expected given that T_1 has been in culture for a much longer period than T_2. The evidence is that particular chromosomes tend to be more highly prone to loss, while others are lost rather rarely, as has been noted in other studies (Lee and Phillips 1988; Doğramaci-Altuntepe et al. 2001). The former group includes chromosomes 4A, 5A, 3B, 7B, 3D, 6D, and the latter chromosomes 3A, 6A, 4B, 5B and 4D (Table 2). As for the chromosomal abnormalities of T_1 and T_2, the frequencies in D-, B-groups were higher than that of the A-group, with the order of D- > B- > A-group in both T_1 and T_2 (Table 2).

Hybrid chromosome number and regeneration ability

Previous studies have indicated that the totipotency of a hybrid cell line was dependent on the number of chromosomes present, so those having a chromosome number close to the bread wheat somatic number of 42 tended to be more fit than those which were either hypo- or hyper-ploid (Xia et al. 1996; Xiang et al. 2003a, 2004). The majority of the regenerants from the wheat/oat fusion had a chromosome number in the range 46–48, far less than the sum of the three fusion parents' chromosomes (93–108). Thus, there must have been a massive and rapid phase of chromosome elimination following the fusion, particularly involving the oat chromosomes (aided by the pre-fusion irradiation treatment), but also involving chromosomes inherited from T_1 and T_2.

Table 4 Chromosome content of a bread wheat/oat somatic hybrid derivative

A-genome chromosome	No. of chromosome	B-genome chromosome	No. of chromosome	D-genome chromosome	No. of chromosome	Abnormal chromosome	No. of chromosome
1A	2	1B	2	1D	2		
2A	3	2B	2	2D	1		
3A	1	3B	3	3D	1		
4A	3	4B	1	4D	2		
5A	4	5B	1	5D	2		
6A	2	6B	1	6D	3		
7A	2	7B	3	7D	1		
		XB	1			X	2
		YB	1			Y	1
Total	17	Total	15	Total	12	Total	3

XB and *YB* unidentified likely B genome chromosome, based on its FISH profile

X, *Y*, unidentified chromosome

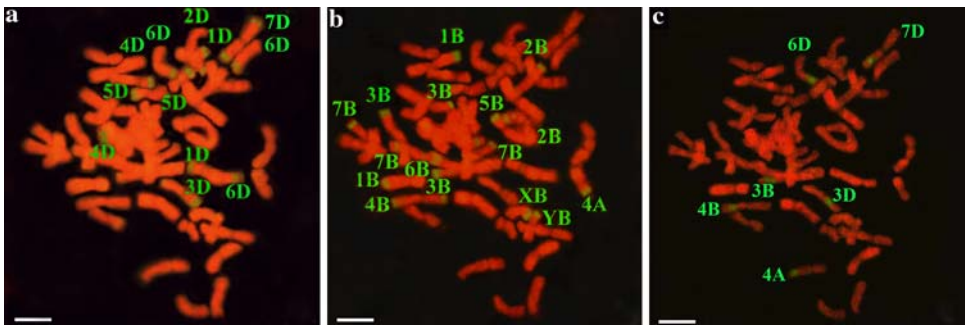

Fig. 4 Sequential FISH and GISH preparations of metaphase chromosomes of a bread wheat/oat somatic hybrid derivative. FISH profiling to identify B (**a**) and D (**b**) genome chromosomes. **c** GISH profile to identify chromosome segments introgressed from oat. *Scale bars* 10 μm

Table 5 Localization of introgressed oat chromatin in a wheat/oat somatic hybrid derivative

Chromosome		Cell number	Relative distance (%) from centromere to the breaking point[a]	Arm ratio
4A	L	27	60.55 ± 11.12	1.45 ± 0.09
3B	S	32	21.63 ± 10.19	1.50 ± 0.13
4B	L	33	29.71 ± 11.25	1.02 ± 0.07
3D	S	38	40.57 ± 12.33	1.46 ± 0.12
6D	L	31	58.58 ± 11.02	1.41 ± 0.13
7D	L	40	27.29 ± 11.57	1.08 ± 0.08

L chromosome long arm; *S* chromosome short arm

[a] The distance from the centromere to the break point/ the length of the arm involved × 100%

Table 6 SSR position on the wheat chromosome of hybrid and the amplification marker

Primer	Location	Band pattern	Relative distance (%) from the centromere	Primer	Location	Band pattern	Relative distance (%) from the centromere[a]
Xgwm369	3AS	P. N	76.36	Xpsp3030	3BS	O	21.21
Xgwm674	3AL	P	3.33	Xgwm165	4BL	P	19.50
Xwmc258	4AL	O	56.00	Xgwm107	4BL	P	3.54
Xwmc262	4AL	P	59.33	Xgwm456	3DS	P	38.1
Xgwm494	6AL	P	3.10	Xgdm98	6DL	P	67.05
Xgwm63	7AL	P	68.97	Xpsp3079	7DL	P	29.31
Xwmc231	3BS	P	11.95	Xgwm428	7DL	P. N	22.24

P both parental alleles; *O* oat allele; *N* novel allele; *T* wheat allele

[a] The distance from the centromere to SSR locus checked/the length of the arm involved × 100%

Genetic complementation of T_1, T_2 and *A. sativa* with hybrid plant regeneration

When bread wheat is pollinated by either *Hordeum bulbosum* or maize, the pollen parent's chromosomes are eliminated during the first few mitotic divisions of the hybrid zygote, a process which has been exploited for the production of dihaploids (Inagaki and Tahir 1990; Chen et al. 1999). Even in compatible wide crosses, which occur in nature and are maintained by polyploidization, a spectrum of genetic and cytological events leads to various modifications (Kashkush et al. 2003; Birchler et al. 2005; James and Reiner 2007; Ma and Gustafson 2008). Thus, it is unsurprising that such events also affect the somatic hybridization process. The T_1 cell line has lost the capacity to regenerate, while T_2 cells remain totipotent (Xiang et al. 2003a, 2004; Xu et al. 2003). T_2 protoplasts cannot divide in vitro, while T_1 cells readily form non-regenerable calli. In combination, these two lines are able to contribute both the ability to grow in vitro and totipotency. Thus, it is plausible to suggest that the loss of totipotency of the T_1 cell line is due to the absence of a critical chromosome(s) and that this absence can be complemented in the fusion nucleus by the contribution of the T_2 line genome, where the critical chromosome(s) is still represented. A similar argument can be made for the ability to divide in in vitro culture. In addition, GISH/FISH pattern indicated the introgression of oat chromatin to the 4AL, 3BS, 4BL, 3DS, 6DL and 7DL of the hybrid wheat (Table 5, Fig. 4). It is interesting that 10 SSR loci near to the introgression position showed the same profile between the hybrid and oat (Table 6). Therefore, we suggested that hybrid plant regenerated through genetic complementation of T_1 and T_2 and oat. Somatic hybrid derivatives have also been recovered from other combinations with wheat, including oat, foxtail millet and maize (Xiang et al. 2003a, 2004; Xu et al. 2003), which may be explained by the genetic

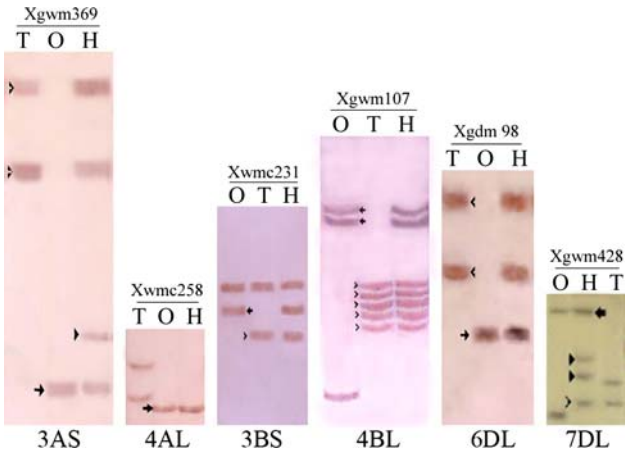

Fig. 5 SSR profiles of cv. Jinan 177 (*lane T*), oat (*lane O*) and a bread wheat/oat somatic hybrid derivative (*lane H*), based on primers directed to loci on wheat chromosome arms 3AS, 4AL, 3BS, 4BL, 6DL and 7DL. Oat alleles indicated by an *arrow*, wheat alleles by a *thin arrowhead*, novel alleles by a *full arrowhead*

complementation between the T_1 and T_2 cell lines and the donor species. But no hybrid progenies were produced in these plants from the "Triple parents" (Xiang et al. 2003a, 2004; Xu et al. 2003). In our early reports, hybrid cells from high capacity for regeneration suspension of wheat cv. Jinan 177 with wheatgrass DNA were fertile and heredity stable (Wang et al. 2005). Thus, we suggest that besides the genetic complementation, some other factors also affect the fertileness and genetic stability of these distant hybrid plants. This is worthy of further research (Fig. 5).

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